IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	:	09/230,463	Confirmation No.: 4323
Applicant	:	David WYNICK	
Filed	:	July 26, 1999	
Title	:	Galanin	
TC/Art Unit	:	1647	
Examiner:	:	Stephen GUCKER	
Docket No.	:	68150.000002	
Customer No.	:	**21967**	

Commissioner for Patents
P.O. Box 1450
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SECOND DECLARATION UNDER 37 C.F.R. § 1.132

Sir,

I, David Wynick, declare that:

1. I have received a BSc (Hon) in Biochemistry from London University in 1980; MBBS from London University in 1983; MD from London University in 1992; and PhD from the Open University in 1997.

2. I am Professsor of Molecular Medicine at, and am employed by The University of Bristol, United Kingdom. I have been associated with research in the field of neuropeptides and molecular neuroscience for approximately 19 years.

3. My *curriculum vitae* providing details of experience is attached.

4. I am a named inventor of U.S. Patent Application 09/230,463. Based on the academic training and professional experience, I consider myself a person of ordinary skill in the technology of galanin peptides and their biological activity thereof, and I was such a person in 1997 when the instant Application was filed.

5. I have read, and am familiar with, the following documents:

   a. U.S. Patent Application No. 09/230,463 ("the '463 application");

   b. The Final Office Action mailed March 14, 2006, in the '463 application ("Office Action");

c. Luo *et al.* (March 1995) "The effects of pretreatment with tachykinin antagonists and galanin on the development of spinal cord hyperexcitability following sciatic nerve section in the rat." Neuropeptides **28**(3): 161-6 ("Luo *et al.*");

d. Zhang *et al.* (May 1993) "Effect of peripheral nerve cut on neuropeptides in dorsal root ganglia and the spinal cord of monkey with special reference to galanin." J Neurocytol. **22**(5): 342-81 ("Zhang *et al.*");

e. Declaration by Professor Dickenson filed November 9, 2005 ("Dickenson Declaration") (**EXHIBIT A**);

f. First Declaration by Professor Zigmond signed June 28, 2004 ("First Zigmond Declaration) (**EXHIBIT B**); and

g. Second Declaration by Professor Zigmond signed June 1, 2005 ("Second Zigmond Declaration") (**EXHIBIT C**).

6. I have been asked to comment on whether Luo *et al.* teaches or suggests each and every limitation of the claims.

7. I have been asked to comment on whether Zhang *et al.* teaches or suggests each and every limitation of the claims.

8. I understand that this Declaration will be submitted in response to the Office Action.

9. The main finding of the '463 application is that a galanin agonist would be an effective treatment to stimulate regeneration of injured sensory neurons.

10. At the time the the '463 application was filed there was no prior art in this field on this subject.

11. The invention is drawn to a method for the treatment of peripheral sensory neuropathy comprising administering an amount of a galanin agonist effective to treat peripheral sensory neuropathy, wherein said peripheral neuropathy was treated by nerve regeneration. First Zigmond Declaration at ¶ 9.

12. Further, our findings as presented in the '463 application, are both unexpected and significant.

13. The '463 application includes the surprising result that nerve regeneration is stimulated by galanin in a dose-dependent manner.

14. This effect is statistically significant as reported in the specification of the '463 application at page 10 line 17 to page 11 line 15 and Figures 8-9.

15. I hereby declare that Luo *et al.* does not teach or suggest the limitations of the invention for at least the following reasons.

16. New claims 27 and 37 include distance limitations which can not be met by Lou *et al.* because the reference does not disclose any distance measurements of sensory nerve axon regeneration.

17. New claims 30 and 40 limitations require an increase in the rate of regeneration of sensory nerve axons which is not disclosed by Luo *et al.*

18. Luo *et al.* deals exclusively with neuropathic pain behavior, not with peripheral nerve damage such as peripheral sensory neuropathy— peripheral nerve damage is in no way synonymous with chronic neuropathic pain. Dickenson Declaration at ¶ 18; First Zigmond Declaration at ¶ 7.

19. Thus, Luo *et al.* and the instant claims cover two distinct aspects of therapy. First Zigmond Declaration at ¶ 8.

20. Further, Luo *et al.* discloses the administration of galanin directly to the spinal cord, the central nervous system, versus the instant claims which are drawn to peripheral sensory neuropathy which is damage in the peripheral nervous system. First Zigmond Declaration at ¶ 7.

21. The mechanism by which galanin rapidly alters pain activity is most likely by direct modulation of the spinal cord neuronal firing rather than at the level of the dorsal root ganglion (DRG). Second Zigmond Declaration at ¶ 8.

22. For the galanin administered in Luo *et al.* during the 90 minute period before the animals were sacrificed to effect regeneration, the peptide would have to gain access to the cell bodies in the DRG, since this is where the intra-cellular pro-regenerative machinery resides. Second Zigmond Declaration at ¶ 9.

23. It is clear that galanin could not have reached the DRG in the 90 minutes after it was administered into the lining of the spinal cord. Second Zigmond Declaration at ¶ 10.

24. Galanin could not, therefore, have even begun to affect regeneration in the DRG cell bodies by the time the experiment was terminated. Second Zigmond Declaration at ¶ 12.

25. I hereby declare that Zhang *et al.* does not teach or suggest the limitations of the invention for at least the following reasons.

26. New claims 27 and 37 include distance limitations which can not be met by Zhang *et al.* because the reference does not disclose or suggest any distance measurements of sensory nerve axon regeneration.

27. New claims 30 and 40 include limitations requiring the increase in the rate of regeneration of sensory nerve axons which was not disclosed or suggested by Zhang *et al.*

28. Zhang *et al.* only contains suggestion that "galanin agonists should represent new pharmacological tools to suppress chronic pain" and not with peripheral nerve damage such as peripheral sensory neuropathy. Id. at 375.

29. The treatment of peripheral nerve damage is in no way synonymous with chronic neuropathic pain. First Zigmond Declaration at ¶ 7.

30. Zhang *et al.* and the instant claims cover two distinct phenomena and even though pain and regeneration may both be treated and effected, respectively by galanin, they remain different processes.

31. Most, if not all, of the anti-pain drugs in current use, act at the level of the spinal cord and brain to reduce electrical and chemical excitability and thus reduce pain transmission. Dickenson Declaration at ¶¶ 16-19.

32. In contrast, drugs that stimulate nerve regeneration do so at the level of the dorsal root ganglion (DRG) and/or the site of nerve injury. Dickenson Declaration at ¶ 19.

33. Therefore, the actions of galanin when administered directly to the spinal cord (central nervous system) to inhibit chronic pain behaviour, in no way implies or predicts regeneration at the level of the sciatic nerve (peripheral nervous system). Dickenson Declaration at ¶ 20; First Zigmond Declaration at ¶ 7.

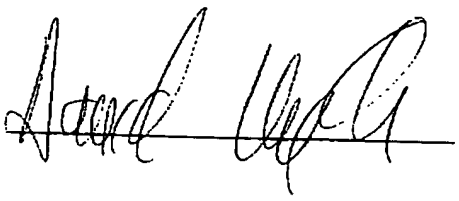
34. Zhang *et al.* suggests that galanin might be administered to primates (including humans) to suppress chronic pain. Id. at 375.

35. Nothing in Zhang *et al.* provides any expectation that galanin would have any effect other than analgesia. Dickenson Declaration at ¶ 20; Second Zigmond Declaration at ¶ 14.

36. The undersigned acknowledges that willful false statements and the like are punishable by fine or imprisonment, or both (18 U.S.C. § 1001), and may jeopardize the validity of the application or any patent issuing thereon. The undersigned declares further that all statements made herein of his/her own knowledge are true and that all statements made on information and belief are believed to be true.

37. I declare under penalty of perjury that the foregoing is true and correct.

Professor David WYNICK

Executed on 13th Sept 2006. Declarant's Signature: 

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1. PERSONAL INFORMATION

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1.1 Academic Qualifications

Intercalated BSc (Hon) Biochemistry, London University	1980
MBBS, Middlesex Hospital, London University	1983
Member of The Royal College of Physicians (MRCP, UK)	1986
MD, London University	1992
PhD, Open University	1997
FRCP	1998

1.2 Present Appointment

Professor of Molecular Medicine and Honorary Consultant Physician UBHT
Department of Clinical Sciences, South Bristol
Bristol University — 2000- present

1.3 Previous Appointments

Senior Lecturer and Honorary Consultant Physician UBHT,
University Research Centre for Neuroendocrinology
Department of Clinical Medicine
Bristol University — 1995-00

Senior Lecturer and Honorary Consultant Physician,
The Royal Postgraduate Medical School
Hammersmith Hospital — 1994-95

Medical Research Council Clinician Scientist,
The Royal Postgraduate Medical School and
The National Institute for Medical Research — 1992-94

Rotational Registrar, General Medicine/Endocrinology
Ealing and Hammersmith Hospitals — 1990-92

Wellcome Trust Training Fellow,
Honorary Registrar, Department of Medicine
The Royal Postgraduate Medical School — 1987-90

Senior House Officer, Thoracic Medicine, The London Chest Hospital	1986-87
Senior House Officer, Cardiology, Hammersmith Hospital	1986
Senior House Officer, Neurology, Middlesex and University College Hospitals	1985-86
Senior House Officer, Medical Rotation, Whittington Hospital	1984-85

2. RESEARCH PUBLICATIONS

Full Publications in Academic Journals (peer reviewed)
I have used asterisks to indicate my intellectual contribution to each paper.

* Substantial contribution (>50% total input)
** Major contribution (> 75% total input)

1. Bender, D. A. & Wynick, D. Inhibition of kynureninase by oestrone sulphate: an alternative explanation for abnormal results of Tryptophan load tests in women receiving oestrogenic steroids (1980) *Br. J. Nutr.* **45, 269-275.

2. Bender, D. A., Magboul, B. I. & Wynick, D. Probable mechanisms of regulation of the utilisation of dietary Tryptophan, Nicotinamide, and Nicotinic acid as precursors of Nicotinamide nucleotides in the rat (1981) *Br. J. Nutr.* **48, 119-127.

3. Wynick, D. & Jessop, J. J. A survey of cost awareness amongst hospital medical staff (1984) *Health Trends* **17, 24.

4. Wynick, D. & Bloom, S. R. Effect of calcitonin in patients with malignant metastatic islet cell tumours (1987) *Br. Med. J.* **295, 1346.

5. Wynick, D., Williams, S. J. & Bloom, S. R. Symptomatic secondary hormone syndromes in patients with established malignant pancreatic endocrine tumors (1988) *N. Engl. J. Med.* **319, 605-607.

6. Wynick, D., Anderson, J. V., Williams; S. J. & Bloom, S. R. Resistance of metastatic pancreatic endocrine tumours after long- term treatment with the somatostatin analogue octreotide (SMS 201- 995) (1989) *Clin. Endocrinol. Oxf.* **30, 385-388.

7. Nicholls, J., Wynick, D., Domin, J., Sandler, L. M. & Bloom, S. R. Pharmacokinetics of the long-acting somatostatin analogue octreotide (SMS 201-995) in acromegaly (1990) *Clin. Endocrinol. Oxf.* **32, 545-550.

8. Wynick, D. & Bloom, S. R. Magnetic bead separation of anterior pituitary cells (1990) *Neuroendocrinology* **52, 560-565.

9. Wynick, D., Dollery, C. T., Bloom, S. R., Polak, J. M. & Lynn, J. A. Gastrinoma syndrome in multiple endocrine neoplasia (1990) *Br. Med. J.* **301, 489-490.

10. Wynick, D., Ratcliffe, W. A., Heath, D. A., Ball, S., Barnard, M. & Bloom, S. R. Treatment of a malignant pancreatic endocrine tumour secreting parathyroid hormone related protein (1990) *Br. Med. J.* **300, 1314-1315.

11. Wynick, D., Critchley, R., Venetikou, M. S., Burrin, J. M. & Bloom, S. R. Purification of functional lactotrophs and somatotrophs from female rats using fluorescence-activated cell sorting (1990) *J. Endocrinol.* **126, 269-274.

12. Wynick, D., Venetikou, M. S., Critchley, R., Burrin, J. M. & Bloom, S. R. Flow cytometric analysis of functional anterior pituitary cells from female rats (1990) *J. Endocrinol.* **126, 261-268.

13. Wynick, D., Rees, A. J., Waxman, J., Bloom, S. R. & Dollery, C. T. Medical registrar training (1991) *Br. Med. J.* **302, 595.

14. Wynick, D., Smith, D. M., Ghatei, M., Akinsanya, K., Bhogal, R., Purkiss, P., Byfield, P., Yanaihara, N. & Bloom, S. R. Characterization of a high-affinity galanin receptor in the rat anterior pituitary: absence of biological effect and reduced membrane binding of the antagonist M15 differentiate it from the brain/gut receptor (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90, 4231-4235.

15. Wynick, D., Hammond, P. J., Akinsanya, K. O. & Bloom, S. R. Galanin regulates basal and oestrogen-stimulated lactotroph function (1993) *Nature* **364, 529-532.

16. Hammond, P. J., Smith, D. M., Akinsanya, K. O., Mufti, W. A., Wynick, D. & Bloom, S. R. Signalling pathways mediating secretory and mitogenic responses to galanin and pituitary adenylate cyclase-activating polypeptide in the 235-1 clonal rat lactotroph cell line (1996) *J. Neuroendocrinol.* **8, 457-464.

17. Wynick, D., Small, C. J., Bacon, A., Holmes, F. E., Norman, M., Ormandy, C. J., Kilic, E., Kerr, N. C. H., Ghatei, M., Talamantes, F. *et al.* Galanin regulates prolactin release and lactotroph proliferation (1998) *Proc. Natl. Acad. Sci. U. S. A* **95, 12671-12676.

18. Kerr, B. J., Cafferty, W. B. J., Gupta, Y. K., Bacon, A., Wynick, D., McMahon, S. B. & Thompson, S. W. N. Galanin knockout mice reveal nociceptive deficits following peripheral nerve injury (2000) *Eur J Neurosci* **12, 793-802.

*19. Mazarati, A. M., Hohman, J., Bacon, A., Liu, H., Sankar, R., Steiner, R. A., Wynick, D. & Wasterlain, C. G. Galanin modulation of hippocampal excitability and seizures. (2000) *J Neurosci* **20**, 6276-6281.

20. Holmes, F. E., Mahoney, S., King, V. R., Bacon, A., Pachnis, V., Curtis, R., Priestley, J. V., and Wynick, D. Targeted disruption of the galanin gene reduces the number of sensory neurons and their regenerative capacity. (2000) *Proc Natl Acad Sci U S A* **97, 11536-11568. (Direct submission via Track II).

21. O'Meara, G., Coumis, U., Shuang, Y. M., Kehr, J., Mahoney, S., Bacon, A., Allen, S. J., Holmes, F. E., Kahl, U., Wang, F. H., Kearns, I. R., Ogren, S. O., Dawbarn, D., Mufson, E. J., Davies, C. H., Dawson, G. R., and Wynick, D. Galanin regulates the post-natal developmental survival of a sub-set of basal forebrain cholinergic neurons. (2000) *Proc Natl Acad Sci U S A* **97, 11569-11574. (Direct submission via Track II).

22. Kerr, N. C. H., Holmes, F. E., and Wynick, D. Galanin-like peptide (GALP) is expressed in the hypothalamus and pituitary but not in the dorsal root ganglion (2000) *Neuroreport.* **11, 3909-3913.

23. Wynick, D. (2001) The role of galanin as a multi-functional neuropeptide in the nervous system. *Current Opinion in Pharmacology* **1, 73-77.

24. Perez, S. E., Wynick, D., Steiner, R. A., and Mufson, E. J. (2001) Distribution of galaninergic immunoreactive in the brain of the mouse. *J Comp Neurol* **434, 158-185.

*25. Kehr, J., Yoshitake, T., Wang, F. H., Wynick, D., Holmberg, H., Lendahl, U., Bartfai, T., Yamaguchi, M., Hokfelt, T., and Ogren, S. O. (2001) Microdialysis in freely moving mice: determination of acetylcholine, serotonin and noradrenaline release in galanin transgenic mice. *Journal Of Neuroscience Methods* **109,** 71-80.

26. Kerr, B. J., Thompson, S. W. N., Wynick, D., and McMahon, S. B. (2001) Endogenous galanin is required for the full expression of central sensitization following peripheral nerve injury. *Neuroreport* **12, 3331-3334.

27. Kerr, B. J., Gupta, Y. K., Pope, R. M., Thompson, S. W. N., Wynick, D., and McMahon, S. B. (2001) Endogenous galanin potentiates spinal nociceptive processing following inflammation. *Pain* **93, 267-277.

**28. Bacon, A., Holmes, F. E., Small, C. J., Ghatei, M., Mahoney, S., Bloom, S. R., and Wynick, D. (2002) Transgenic over-expression of galanin in injured primary sensory neurons. *Neuroreport* 13, 2129-2132.

**29. Mahoney, S., Hosking, R., Farrant, S., Holmes, F. E., Jacoby, A. S., Shine, J., Iismaa, T. P., Scott, M. K., Schmidt, R., and Wynick, D. (2003) Galanin plays a key role in neurite outgrowth from adult sensory neurons via activation of the second galanin receptor and protein kinase C. *J Neurosci* 23(2), 416-421.

*30. Massey, P. V., Warburton, E. C., Wynick, D., Brown, M. W., and Bashir, Z. I. (2003). Galanin regulates spatial memory but not visual recognition memory or synaptic plasticity in perirhinal cortex. *Neuropharmacology* 44(1), 40-48.

**31. Holmes, F. E., Bacon, A., Pope, R. J., Vanderplank, P. A., Kerr, N. C., Sukumaran, M., Pachnis, V., and Wynick, D. (2003) Transgenic overexpression of galanin in the dorsal root ganglia modulates pain-related behaviour. *Proc Natl Acad Sci U S A* 100(10), 6180-6185. (Direct submission via Track II).

**32. Naylor, M. J., Ginsburg, E., Iismaa, T. P., Vonderhaar, B. K., Wynick, D., and Ormandy, C. J. (2003) The neuropeptide galanin augments lobuloalveolar development. *Journal Of Biological Chemistry*. 278(31):29145-52

*33. Zachariou, V., Brunzell, D. H., Stedman, D. B., Hawes, J., Bartfai, T., Wynick, D., Langel, U., and Picciotto, M. R. (2003) The neuropeptide galanin modulates behavioral and neurochemical signs of opiate withdrawal. *Proc Natl Acad Sci U S A* 100: 9028-9033. (Direct submission via Track II).

**34. Hohmann, J. G., Krasnow, S. M., Teklemichael, D. N., Clifton, D. K., Wynick, D., and Steiner, R. A. (2003) Neuroendocrine profiles in galanin-overexpressing and knockout mice. *Neuroendocrinology* 77(6), 354-366.

**35. Mahoney, S. A., Hosking, R., and Wynick, D. (2003). The galanin antagonist M35 has intrinsic agonistic activity in the dorsal root ganglion. *Neuroreport.* 14(12):1649-52.

*36. Ahren, B., Pacini, G., Wynick, D., Wierup, N., and Sundler, F. (2004). Loss-of-Function Mutation of the Galanin Gene Is Associated with Perturbed Islet Function in Mice. *Endocrinology* 145(7), 3190-3196.

**37. Elliott-Hunt, C. R., Marsh, B., Bacon, A., Pope, R., Vanderplank, P., and Wynick, D. (2004). Galanin acts as a neuroprotective factor to the hippocampus. *Proc Natl Acad Sci USA*. 101, 5105-5110. (Direct submission via Track II).

*38. Hohmann, J. G., Teklemichael, D. N., Weinshenker, D., Wynick, D., Clifton, D. K., and Steiner, R. A. (2004). Obesity and endocrine dysfunction in mice with deletions of both neuropeptide Y and galanin. *Molecular & Cellular Biology* 24(7), 2978-2985.

**39. Kerr, N. C. H., Holmes, F. E., and Wynick, D. (2004). Novel isoforms of the sodium channels $Na_v1.8$ and $Na_v1.5$ are produced by a conserved mechanism in mouse and rat. *Journal Of Biological Chemistry* 279, 24826-24833.

**40. Hobson, S. A., Holmes, F. E., Kerr, N. C. H., Pope, R. J., and Wynick, D. Mice deficient for galanin receptor 2 have decreased neurite outgrowth from adult sensory neurons and impaired pain-like behaviour. Journal of Neurochemistry. 2006 (In Press).

2.4.2 Book Chapters and Review Articles

**1. Wynick, D. & Bloom, S. R. Endocrine tumours of the gastro-entero-pancreatic system (1988) *Cancer Topics* 7, 26-28.

**2. Wynick, D., Polak, J. & Bloom, S. R. (1988) in *Gastrointestinal APUDomas*, ed. Buchanan, K. pp. 3-9.

3. Wynick, D., Polak, J. M. & Bloom, S. R. Somatostatin and its analogues in the therapy of gastrointestinal disease (1989) *Pharmacol. Ther.* **41, 353-370.

4. Wynick, D. & Bloom, S. R. Endocrine causes of diarrhoea (1989) *Med Int* **64, 2663.

5. Wynick, D. & Bloom, S. R. Sandostatin and the Hammersmith experience (1990) *Digestion* **45, 5-9.

6. Wynick, D. & Bloom, S. R. The use of the long-acting somatostatin analog octreotide in the treatment of gut neuroendocrine tumors (1991) *J. Clin. Endocrinol. Metab.* **73, 1-3.

**7. Wynick, D. & Bloom, S. R. (1992) in *Clinical Endocrinology*, ed. Grossman, A. (Blackwell Scientific Publications, Oxford), pp. 502-511.

8. Wynick, D., Hammond, P. J. & Bloom, S. R. The glucagonoma syndrome (1993) *Clin. Dermatol.* **11, 93-97.

*9. Hammond, P. J., Gilbey, S. G., Wynick, D. & Bloom, S. R. (1993) in *Endocrine Tumors*, eds. Mazzaferri, E. L. & Samaan, N. A. (Blackwell Scientific Publications, Boston), pp. 457-483.

*10. Hammond, P. J., Wynick, D. & Bloom, S. R. (1993) in *Gastroenterology: clinical science and practice*, eds. Bouchier, I. A. D., Allan, R. N., Hodson, H. J. F., & Keighley, M. R. B. (W.B. Saunders Company, London), pp. 1656-1668.

**11. Wynick, D. & Bloom, S. R. (1993) in *Surgical Endocrinology*, ed. Lynn, J. (Butterworth Heinemann, Oxford), pp. 487-493.

**12. Wynick, D., Polak, J. M. & Bloom, S. R. (1993) in *Oxford Textbook of Oncology*.

*13. Gilbey, S., Wynick, D. & Bloom, S. R. (1994) in *Joslin's Dibetes Mellitus*, eds. Kahn, C. R. & Weir, G. C. (Lea & Febiger, Philadelphia), pp. 1000-1022.

*14. Akinsanya, K. O., Griffiths, A. J., Wang, Z., Wynick, D. & Bloom, S. R. (1995) in *Gastrointestinal Tract and Endocrine System*, eds. Singer, M. V., Ziegler, R., & Rohr, G. (Kluwer Academic Publishers, Dordrecht Boston London), pp. 95-112.

15. Wynick, D., Small, C. J., Bloom, S. R. & Pachnis, V. Targeted disruption of the murine galanin gene (1998) *Ann. N. Y. Acad. Sci.* **863, 22-47.

16. Wynick, D., Thompson, S. W. N., and McMahon, S. B. Galanin and pain (2001) *Progress in Neurobiology* **1, 73-77.

**17. Wynick, D. and Bacon, A. (2002). Targeted disruption of galanin: new insights from knock-out studies. *Neuropeptides* 36(2-3), 132-144.

GALANIN

This invention relates to galanin, including analogues thereof and its uses.

Galanin is a 29 amino acid neuropeptide which was first isolated from porcine intestine in 1983. Subsequently, the cDNA for galanin was cloned from a rat anterior pituitary library in 1987. Nucleotide and amino-acid sequence analysis suggests that galanin is unrelated to any of the other known families of regulatory peptides, and remains the only member of its family. The N-terminal portion of galanin is highly conserved between species, there being variation in the C-terminal portion.

Galanin has a widespread distribution in the peripheral and central nervous systems. gut and pancreas. It is found in highest levels in the median eminence of hypothalamus and in the pituitary W092/12997 (General Hospital Corporation), published in 1992, discloses the sequence of human galanin. There is a discussion of studies by other workers involving the administration of rat galanin or its N-terminal fragments to augment the effect of morphine and this patent application suggests that galanin can be expected to exhibit analgesic effects such that it may be administered alone or in combination with other analgesics. The application claims the use of galanin or its analogues in the treatment of pain and the use of galanin antagonists in the treatment of certain other conditions.

W092/20709 (Astra AB) discloses a number of putative galanin antagonists. The antagonists which are described are all based on the first 12 amino acids of galanin followed by partial sequences of other peptides i.e. chimeric peptides. Some may be agonists, some antagonists and some may be both depending on the receptor subtype. The application discloses that the antagonists may be useful for treatment of insulin-, growth hormone-, acetyl choline-, dopamine-, Substance P-, Somatostatin-, and noradrenaline-related conditions including endocrinology, food intake, neurology and psychiatry, Alzheimer's type dementia, analgesia, intestinal disease. The application discloses the results of studies using some of the antagonists described therein on various effects such as galanin inhibition of glucose stimulated insulin release; galanin induced inhibition of scopolamine induced ACh hippocampal release; galanin induced facilitation of the flexor reflex; the displacement of bound iodinated galanin in membrane binding studies. There is a suggestion in the application that the antagonists may be indicated for analgesia but there is no disclosure in the application of results to this effect.

Approximately 2-4% of the Western population suffer from diabetes mellitus and, of those people, 10-15% suffer from chronic pain and numbness in their extremities-termed "painful neuropathy". Present techniques for management of painful neuropathy are inadequate.

Alzheimer's disease is a major cause of morbidity worldwide the disease being characterised by loss of memory and personality changes. At an anatomical level there is a major decrease in the number of cholinergic nerves in the hippocampus, which is the main area of the brain thought to process and store memories. Previous work has shown

that galanin is also expressed in these hippocampal nerves and the levels of galanin are two fold elevated in the brains of patients with Alzheimer's disease.

The present invention relates to the generation of a mouse with targeted disruption of the galanin gene; experiments using the mouse, and the implication of the results of those experiments for the treatment of disease. In particular, the invention relates to the generation of a mutant mouse carrying a loss-of-function germ-line mutation of the galanin locus. The inactivating mutation has been introduced into the mouse genome utilising targeted mutagenesis in embryonic stem cells by homologous recombination. The mutation, when bred to homozygosity on the inbred 129sv background, affects feeding behaviour, lactation and pain sensitivity. The mutation may also affect memory and behaviour, sexual reproduction and fertility and insulin secretion with resultant changes in circulating blood glucose levels.

According to first aspect of the invention there is provided the use of a galanin agonist in the preparation of a medicament for the treatment of nerve damage.

According to a second aspect of the invention there is provided a method of healing, preferably repairing, nerve damage in a subject comprising administering to the subject a galanin agonist.

According to another aspect of the invention there is provided a method of treatment of Alzheimer's disease and related diseases and conditions, the method comprising administering a galanin agonist to a subject.

In a further aspect of the invention, there is provided a method of improving memory, enhancing memory and improving cognitive function, comprising administering a galanin agonist to a subject. Advantageously, such treatment may be used in the treatment of restoring memory after injury or trauma.

According to a further aspect of the invention there is provided the use of a galanin antagonist in the preparation of a medicament for the suppression of lactation and also a method of suppressing lactation in a mammal, the method comprising administering a galanin antagonist to that mammal.

According to another aspect of the invention there is provided a composition comprising a galanin antagonist for the treatment of prolactinoma in a mammal and also the use of a galanin antagonist in the preparation of a medicament for the treatment of prolactinoma and a method of treating prolactinoma in a mammal suffering from prolactinoma, the method comprising administering a galanin antagonist to that mammal.

The invention further provides galanin agonists suitable for use in the treatment of Alzheimer's disease, related diseases and conditions and in the improvement of memory and cognitive function. Also, the invention provides the use of a galanin agonist in the preparation of a medicament for the treatment of Alzheimer's disease and related diseases and conditions, and in enhancing memory and cognitive function.

According to a further aspect of the invention there is provided an analgesic composition comprising a galanin antagonist and, in addition, the use of a galanin antagonist in the preparation of a medicament for the treatment of pain.

According to a further aspect of the invention there is provided a method of suppressing pain in a mammal, the method comprising administering a galanin antagonist to that mammal and, in addition, the use of a galanin antagonist in the preparation of a medicament for the treatment of painful neuropathy.

According to a further aspect of the invention there is provided an appetite suppressant composition comprising a galanin antagonist and, in addition, the use of a galanin antagonist in the preparation of a medicament for the suppression of appetite. This aspect of the invention also provides a method of suppressing appetite in a mammal, the method comprising administering a galanin antagonist to that mammal.

According to a further aspect of the invention there is provided an anaesthetic composition comprising a galanin antagonist and, in addition, the use of a galanin antagonist in the preparation of an anaesthetic composition. This aspect of the invention also provides a method of anaesthetising a mammal, the method comprising administering a galanin antagonist to that mammal.

According to a further aspect of the invention there is provided a mammal, preferably a rodent, which lacks a functional galanin gene. The term "galanin" embraces all known galanins including, for example, human, rat, murine and porcine galanin and also analogues of galanin having the biological activity of galanin. The galanin gene may have been inactivated by at least partial deletion of the galanin gene sequence between the BamHI and Bg12 restriction sites indicated by asterisks in the accompanying Fig. 3. Where the mammal is a rodent, it is preferably a mouse. Other mammals such as sheep and rats are contemplated.

According to another aspect of the invention there is provided tissue, cells and cell lines derived from the mammal in accordance with the first aspect of the invention. Preferably, the tissue, cells or cell lines include cells from pancreas, pituitary, cortex, dorsal root ganglia, or are derived from such cells.

The mammal or tissue, cells and cell lines of the invention may be used in an assay to study one or more biological effects of galanin. The biological effect may be selected from, for example, prolactin secretion, appetite, memory, behaviour, pain, autotomy following axotomy, growth or the repair of nerve damage.

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings Figures 1 to 16 in which:

Fig. 1 illustrates the genomic structure of mouse galanin;

Fig. 2 illustrates the targeting vector used in producing the rodent of the invention;

Fig. 3 illustrates the specific recombination event in the production of the rodent in accordance with the invention;

Fig. 4 illustrates the genotype of the progeny determined using Southern blotting and by PCR demonstrating identical results from the same litter derived from a mating of two heterozygote animals;

Fig. 5 illustrates the weight gain and final body weights of wild-type and mutant animals over the first 8 weeks of life;

Fig. 6 illustrates results of experiments on behavioural responses of intact adult mice to thermal and mechanical stimulation;

Fig. 7 illustrates the effect of galanin inactivation on autotomy behaviour after sciatic nerve section;

Fig. 8 illustrates the effect of galanin inactivation on short term regeneration of sensory neurons;

Fig. 9 illustrates the effect of galanin inactivation on long term regeneration of sensory neurons;

Fig 10 illustrates expression of an exon 6-specific riboprobe to study the distribution of galaninergic neurons in the brain and dorsal root ganglion of wildtype and mutant mice;

Fig. 11 illustrates the effect of galanin inactivation on anterior pituitary prolactin content;

Fig. 12 illustrates the effect of galanin inactivation on anterior pituitary thyroid stimulating hormone con tent;

Fig. 13 illustrates the effect of galanin inactivation on anterior pituitary growth hormone content;

Fig. 14 illustrates the effect of galanin inactivation on anterior pituitary luteinizing hormone content;

Fig 15 illustrates the effects of galanin inactivation on the generation of long term potentiation in the stratum radiatum area of the hippocampus; and

Fig 16 illustrates the effects of galanin inactivation on the generation of long term potentiation in the stratum oriens area of the hippocampus.

To generate a mouse knockout, that is the introduction into the mouse genome of either a loss- or gain-of-function mutation of a specific gene locus (according to the procedure described in Kuehn, M. R. *et al* Nature. 1987; **326:** 295-8; Thomas, K. R. and Capecchi, M. R. Nature. 1986; **324:** 34-8) , entails a number of steps:- (1) the cloning of the mouse

genomic locus of interest; (2) the construction of a targeting vector such that the locus/gene of interest is modified to inactivate or alter its structure and function in some way; (3) introduction of the targeting vector into an embryonic stem cell library and selection and identification of single cell clones in whom the appropriate correct targeting event has taken place and in whom the normal chromosomal number is unchanged; and (4) introduction of such clones into 3.5 day old blastocysts and the resulting chimeric mice mated to wild types of the opposite sex. The resulting offspring demonstrated to carry the mutation are thus heterozygotes and, by appropriate mating, homozygotes for the introduced mutation are bred.

As a first step the murine *galanin* gene was cloned. A mouse genomic library (Ehrich, E. *et al* Gene. 1987; *57:* 229-37) was screened using the full length rat *galanin* cDNA as a probe under high stringency. Two cosmid clones were identified spanning 60Kb around the galanin locus. Using 5' and 3' probes from the rat cDNA a 14 Kb region of DNA containing the entire gene was subcloned and partially sequenced. From the genomic sequence, primers were designed complementary to untranslated exonic regions of the gene. A 630bp fragment was generated by RT-PCR (Kit supplied by INVITROGEN BV, The Netherlands) using adult female whole brain as a source of mRNA. Subsequent sequencing of this fragment demonstrated that mouse and rat galanin are 100% identical at the protein level and 94.8% at the nucleotide level. The genomic structure of the mouse gene (Fig. 1) is identical to that of the rat gene. The gene spans 4.8Kb and consists of six exons. The translation start site (AUG) starts at the first base of exon two, the coding region for galanin extends across exons three and four with the stop codon (UGA) in the middle of exon six.

Using the 14Kb subclone described above, a positive/negative selection targeting vector was constructed (Fig. 2). The mutation introduced removes the first five exons containing the entire coding region of the galanin peptide (Fig. 3).

In Fig. 3: A and B are the sites of the external probes used to screen the ES cells for the appropriate integration of the construct

Neo = neomycin resistance gene
HSV-TK= herpes simplex virus thymidine kinase gene

B=BamHI
E=EcoR1
X=Xho1
Bg=Bgl2

In particular, the targeting vector removes a 3.2Kb stretch of DNA and thus removes the first 5 exons of the galanin gene. The exact sites flanking the stretch of DNA removed are 5' - the Bam HI site 10bp downstream from the transcriptional start site and the 3' site is the Bg12 site in the middle of intron 5. These sites are indicated with asterisks in Fig. 3.

Other sites that could be used are the same 5' site and a differing 3' Xho 1 site in intron 4 which would remove only 2.9Kb of DNA and thus remove only first 4 exons.

This vector was linearised and electroporated into the El4 embryonic stem-cell (ES) line (Hooper, M. et *al* Nature. 1987; 326: 292-5). Restriction mapping of the wildtype locus with BglII generates a 9.3Kb fragment when probed with a 5' external probe (marked **A**, Fig 3), whilst the correctly targeted locus generates a 4.4 Kb fragment. In total, 9 clones were identified in which one allele of the galanin gene was correctly targeted by homologous recombination among 209 double resistant colonies yielding a targeting frequency of 4.3%. These nine clones were karyotyped, confirming euploidy, and injected into 3.5 day old blastocysts from C57BL/6 mice. Germ line transmission of the disrupted galanin locus was obtained from three separate ES cell clones. Genotype of the progeny was determined using Southern blotting and by PCR (Fig 4 demonstrates identical results obtained by Southern blotting and PCR screening on the same litter derived from a mating of two heterozygotes). The mutation has been bred to homozygosity on the in-bred 129sv strain and all data presented is from mice on this background.

1. Results of genotype analysis of live births are in the expected ratio predicted by Mendelian genetics and the sex ratio is 1: 1. Galanin levels were measured by radioimmunoassay and immunocytochemistry in areas previously demonstrated to express galanin at high levels and include brain, pituitary, spinal cord, dorsal root ganglion, stomach, small intestine and uterus. Galanin levels in heterozygotes for the deletion were 50% of wild type controls whilst Galanin levels in the homozygotes for the deletion were undetectable in all cases.

A comparison of levels of galanin expression between wild type, heterozygote and mutant mice in several body tissues is shown in Table 1.

Table 1

Genotype	Cortex	Hypothal-amus	Anterior Pituitary	Stomach	Duodenum	Ileum
+/+	5.78±0.33	110.34±7.81	0.42±0.07	27.46±1.91	122.90±11.60	267.43±13.46
+/-	2.91±0.21	53.82±3.76	0.21±0.04	13.8±0.83	68.36±5.67	125.87±7.55
-/-	UD	UD	UD	UD	UD	UD

All values are mean galanin-LI pmol/g ± SEM, other than the female anterior pituitary which is expressed as pmol/gland ± SEM. UD=Undetectable

It will be seen that galanin was not detected in any of the tissues tested in the homozygous mutant mouse, and decreased by 50% in the heterozygous mutant mouse.

2. Although the mutant animals grow normally after weaning compared to their wild type litter mates (Fig 5) and achieve equal adult body weights, the same is not the case if

the animals are weaned two days early. At PI9 (i.e 19 days *postpartum)* galanin would appear to be vital for the development of appetite for solids, if the animals are weaned at this point the mutants die within 48h. of starvation. Post mortem findings reveal a complete absence of food in the stomach or small bowel. Clearly this is a major finding since very little is known about the normal regulation of appetite in the peri-weaning period. The mice of the invention are useful in studies on the expression of other neuropeptides known to regulate appetite (including leptin, neuropeptide Y, CCK, CRF and GLP-I).

3. The behavioural responses of intact adult mice to thermal and mechanical stimulation was tested. Responses to noxious thermal stimulus were measured using the Hargreaves paw withdrawal test (Hargreaves, K., Dubner, R., Brown, F., Flores, C. & Joris, J. Pain **32**,77-88 1988) and the sensitivity to mechanical stimulation was assessed with Von-Frey hairs (Woolf, C.J., Safieh Garabedian, B., Ma, Q.P., Crilly, P. & Winter, J. Neuroscience **62**, 327-331 1994). No significant differences between homozygous mutants, heterozygotes and wild-type mice in either the withdrawal times in the hot plate test or sensitivity to mechanical stimulation (Fig. 6) were observed. Neuronal function does not appear to be compromised by the absence of galanin at least with respect to the sensory modalities tested.

4. Galanin is thought to play a role in the modulation of spinal cord transmission, particularly after nerve damage (axotomy) where its expression is upregulated during axonal regeneration. The response to axotomy is attenuated in the mutants (-/-) and autotomy fails to occur whilst self-mutilation in the wild type litter mates *(+/+)* is severe and occurs in almost all axotomised control animals (Fig. 7). The finding of hypo-algesia in the knockout mice is striking and unexpected. Previous data from Hökfelt's group in Sweden had suggested that galanin has a bimodal response on spinal cord transmission depending on the dose used.

5. The regenerative abilities of sensory axons in the sciatic nerve were directly measured by the pinch test (Danielsen, N., Kerns, J.lM., Holmquist, B., Zhao, Q., Lundborg, G. & Kanje, M. Brain Res. **681**, 105-108 1995). Following nerve crush, sensory axons regenerate into the distal nerve and can be stimulated by a subsequent nerve pinch, which elicits a reflex abdominal motor response. The foremost regenerating axons are located by pinching consecutive segments of the nerve in a distal to proximal direction until a reflex is observed and the distance from the nerve crush can be calculated.

Regeneration showed a statistically significant reduction of 30-40% in homozygotes compared to wild type mice at 2,4 and 6 days after nerve crush (Fig. 8). Regeneration was intermediate in heterozygous mice but was still significantly different from wild type animals.

To test whether the reduced rate of regeneration in galanin-deficient mice affects functional recovery after a crush injury, we tested a behavioural correlate of regeneration using the toe spreading index (Hoogeveen, J.F., Van Der Kracht, A.H., Wondergem, J.,

Gonzalez Gonzalez, D. & Haveman, J. Neurotoxicology. **14**, 1-7 1993). Rodents spread the toes on their hind feet upon contact with a solid surface, a response which requires sensory innervation. Toe spreading is, therefore, lost after axotomy until sensory axon re-innervation occurs. The toe spreading distance was measured for 6 weeks after unilateral right sciatic nerve crush and compared to the intact contralateral (left) foot. Whilst toe spreading in wild-type mice returned to normal within 3 weeks of sciatic nerve crush, functional regeneration was still incomplete at six weeks in the mutant mice (Fig 9).

6. The decreased regeneration and autotomy in the galanin-deficient mice might be related to the death of neurons following axotomy, especially those neurons which would normally express galanin after injury. To test whether galanin is essential for the survival of neurons during development, we studied the distribution of galaninergic neurons in wild type and mutant mice. Since we were unable to visualise the galaninergic neurons in the mutant animals at the protein level we studied expression of the mRNA using a riboprobe specific for exon six (marked B, see Figure 3). In order to confirm the survival of other populations of galanin expressing neurons, the exon 6-specific riboprobe was used to visualise galaninergic neurons in the hippocampus and the paraventricular nucleus of the hypothalamus of adult wild-type and mutant mice (Fig 10). No differences in expression were observed between the groups suggesting that neuronal development are normal in these animals and not galanin dependent.

We went on to use the exon 6-specific riboprobe to study the distribution of galaninergic neurons in the DRG two weeks after sciatic nerve axotomy. A marked up-regulation in the levels and number of cells expressing galanin was observed in the DRG neurons of wild type mice Fig 10). However, there was no expression in the homozygous galanin-deficient mice, suggesting that galanin is required for these cells to survive axotomy.

These results relating to regeneration and cell survival are particularly significant in that the results indicate that galanin gene is the first gene to affect regeneration of the peripheral nervous system.

Accordingly, the invention contemplates the use of a galanin agonist in the treatment of peripheral sensory neuropathy resulting, for example, from diabetes mellitus or trauma (such as that caused by traffic accidents).

7. Homozygote mutants enter puberty at the same time as their litter mate controls, pregnancy and resulting litter size appeared unaffected. Mutant females, however, are unable to lactate and all pups died of dehydration/starvation unless fostered by wild type mothers. Pituitary prolactin content and secretion is reduced some five fold in pregnant homozygotes (-/-) compared to pregnant wild type (+/+) controls killed 4 days after birth (Fig. 11) but is only 80% of normal in randomly cycling female homozygote mice.

The addition of exogenous oestradiol (0.5μg of 17 β-oestradiol given subcutaneously as a suspension in linseed oil) to rodents has a strong mitogenic effect on pituitary cell number and markedly increases pituitary prolactin content (Fig. 11).

These effects are abolished in the knockout mice, confirming that galanin is crucial to lactotroph growth and to prolactin secretion in the hyperoestrogenised state. These findings coupled with previous data that galanin induces growth of the lactotroph, combine to substantiate the hypothesis that an activating mutation in the pituitary galanin receptor may be responsible for the formation of prolactimas (prolactin secreting pituitary tumours).

Anterior pituitary content for three other hormones was assessed. No differences were found in the content of TSH, GH and LH (figs 12-14) in mutant versus wild type mice. It would be expected that the mutant mouse of the invention would have high insulin and low plasma glucose. Thus galanin antagonists might be of use in treatment of diabetes mellitus.

Galanin may inhibit hypothalamic somatostatin release thus stimulating growth hormone. One would expect the mutant mice to have high levels of somatostatin, low GH and to be small. Thus galanin might be a treatment for idiopathic small stature.

Such changes caused by the mutations to the mouse of the invention as disclosed above have implications for possible treatments of a number of human conditions/diseases using either galanin agonists or antagonists. Such diseases may include:- anorexia, obesity, painful neuropathies, pituitary prolactin secreting tumours, Alzheimer's dementia and diabetes.

8. Galanin has been implicated in the aetiology of Alzheimer's disease. Hippocampal galanin expression is increased in cholinergic neurones as acetylcholine and choline acetyl transferase (ChAT) levels fall. Administration of galanin decreases learning behaviour in a number of mouse models, the converse is also true when galanin antagonists are infused. We measured long term potentiation (LTP) in wild type and mutant mice. LTP is an electrophysiological test where specific nerves in the hippocampus are stimulated by an electric shock: Davies CH, Collingridge GL. *J. Physiol. Lond.* 1996;**496**: 45 1-470; Davies CH, Starkey SJ, Pozza MF, Collingridge GL. GABA *Nature* 1991 ;**349**:609-611. This procedure is done *in-vitro* using brain slices from recently killed animals. Results show that LTP is decreased by 50% in the stratum oriens at the 80 minute time point in the mutants compared to wild-type mice (Fig 16 A vs C). In contrast no difference was found in LTP measured in the stratum radiatum. Galanin is found at high levels in the stratum oriens but NOT in the stratum radiatum. Our data, thus far, demonstrates a decrease in LTP in the mutants implying a decrease in memory and cognition - tests to assess these function are being conducted. These data show that a galanin agonist is useful in the treatment of Alzheimer's disease and associated memory loss with an enhancement in memory and cognition.

CLAIMS

1. The use of a galanin agonist in the preparation of a medicament for the treatment of nerve damage.
2. A method of treating nerve damage in a mammal comprising administering a galanin agonist to that mammal.
3. A method of treating Alzheimer's disease and related diseases and conditions comprising administering a galanin agonist to a subject.
4. The use of a galanin agonist in the preparation of a medicament for the treatment of Alzheimer's disease and related diseases and conditions.
5. A method of improving memory, enhancing memory functions and improving cognitive function, the method comprising administering a galanin agonist to a subject.
6. The use of a galanin agonist in the preparation of a medicament for improving memory and other cognitive functions.
7. A lactation suppression composition comprising a galanin antagonist.
8. The use of a galanin antagonist in the preparation of a medicament for the supression of lactation.
9. A method of suppressing lactation in a mammal, the method comprising administering a galanin to that mammal.
10. A composition comprising a galanin antagonist for the treatment of prolactinoma in a mammal.
11. The use of a galanin antagonist in the preparation of a medicament for the treatment of prolactinoma.
12. A method of treating prolactinoma in a mammal suffering from prolactinoma, the method comprising administering a galanin antagonist to that mammal.
13. An appetite supressant composition comprising a galanin antagonist.
14. The use of a galanin antagonist in the preparation of a medicament for the treatment of appetite, and appetite related disorders.
15. A method of supressing appetite in a mammal, the method comprising administering a galanin antagonist to that mammal.
16. An analgesic composition comprising a galanin antagonist.
17. The use of a galanin antagonist in the preparation of a medicament for the treatment of pain.
18. A method of supressing pain in a mammal, the method comprising administering a galanin antagonist to that mammal.
19. The use of a galanin antagonist in the preparation of a medicament for the treatment of painful neuropathy.
20. An anaesthetic composition comprising a galanin antagonist.
21. The use of a galanin antagonist in the preparation of an anaesthetic composition.
22. A method of anaesthetising a mammal, the method comprising administering a galanin antagonist to that mammal.
23. A transgenic or other genetically modified mammal which lacks a functional galanin gene.
24. A mammal according to claim 23 in which the galanin gene has been inactivated.
25. A mammal according to claim 23 or 24 in which the galanin gene has been inactivated by at least partial deletion.

26. A mammal according to claim 25 in which the portion of the galanin gene between the
Bam HI and Bg12 restriction sites asterisked in Fig. 3 has been deleted.
27. A mammal according to claim 23,24, 25 or 26 which is a rodent.
28. A rodent according to claim 27 which is a mouse.
29. Tissue, cells and cell lines derived from a mammal, rodent or mouse according to any preceding claim.
30. Tissue, cells or cell lines according to claim 29 which are cells from pancreas, pituitary, cortex, dorsal root ganglia or are derived from such cells.
31. The use of a mammal, rodent or mouse according to any one of claims 23 to 28 or tissue cells and cell lines according to claim 29 or 30 in an assay to determine a biological effect of galanin.
32. The use according to claim 31 in which the biological effect is selected from diabetes and insulin secretion, appetite, growth hormone effects, lactation, prolactin over secretion, pain sensitivity, memory, behaviour, sexual reproduction and fertility.

EXHIBIT A
Declaration by Professor Dickenson filed November 9, 2005

ATTORNEY DOCKET NO. 23016.0002US
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Wynick, David	)	Art Unit: 1647
	)	
Application No. 09/230,463	)	Examiner: Gucker, S.
	)	
Filing Date: January 22, 1999	)	Confirmation No. 4323
	)	
For: "GALANIN"	)	

DECLARATION UNDER 37 C.F.R. § 1.132

1. I am Professor of Neuropharmacology in the Department of Pharmacology at University College, London, United Kingdom.

2. I have been associated with teaching and research in the subject of Neurosciences for 20 years and have published approximately 220 peer-reviewed papers and 20 review articles and chapters during this time. Examples of these publications together with details of my education are given in the short version of my *curriculum vitae* which is attached and shown as Exhibit A.

3. The work of my research group relates to the physiology and pharmacology of pain transmission and its modulation, with the aim of helping to improve the clinical management of pain. We study how neuronal activity in sensory pathways alters in different states and how this relates to pharmacological systems. Interest centers on the dorsal horn of the spinal cord where painful information can be modulated by both local and descending controls from the brain. The interactions between pain transmission systems and controlling influences, identification of the different transmitters in the incoming nerves, spinal cord neurones and long pathways descending from the brain, are features of this research. We also attempt to gauge the roles of ion channels, excitatory amino-acids, monoamines and neuropeptides, including galanin, in pain processing. The neural bases underlying prolonged pain are of great importance as are individual differences in pain and the ways in which emotional areas of the brain can influence transmission. The relative involvement of

peripheral nervous activity and the central nervous system in the generation of long lasting pain is another area of our research.

4. I am familiar with the work of David Wynick in the field of galanin and nerve regeneration.

5. I have reviewed the specification of US Patent Application Serial No. 09/230,463 ("the patent application"). I understand the technology described in the patent application. In particular, I have reviewed claim 18 of the patent application and its meaning is clear to me.

6. I have also reviewed the Office Action dated 9th August 2005 ("the Office Action") and the cited passage by Rudinger (taken from "Peptide Hormones", edited by J. A. Parsons & published by University Park Press in 1976). In relation to the objection raised by the Examiner in item 5 of the Office Action, I make the following comments:

7. The knowledge of the skilled person at the priority date of the patent application was far advanced from that represented by the disclosure of Rudinger. This textbook was published in 1976, twenty years before the priority date of the patent application and before the identification of galanin. During that time, the fields of biochemistry, neuroscience and pharmacology had changed a great deal.

8. A person of ordinary skill in the art would have been able, at the priority date (24th July 1996) of the patent application, to identify galanin agonists without undue experimentation. Indeed, the level of skill required would be that of a first year undergraduate in Pharmacology at a reasonable university. At the priority date at least six galanin agonists (in addition to the native full-length neuropeptide) had been identified. These include a number of chimeric ligands (where the N-terminal portion of galanin is fused to another peptide), including M15 [GAL-(1-13)-substance P-(5-11)amide], M35 [galanin-(1-13)-bradykinin-(2-9)-amide], M40 [galanin[1-13]-Pro-Pro-[Ala-Leu]2-Ala amide], and Gal(1-14)-[Abu8]SCY-I.

9. The following studies, using a variety of paradigms, demonstrated that each of the above ligands acts as a galanin agonist. M15 and M35 act as galanin agonists by causing contraction of jejunal muscle strips, and relaxing dispersed smooth muscle

cells from the rat small bowel (Gu *et al.* (1993) J. Pharmacol. Exp. Ther. **266** 912-918). Similarly, M15 acts as a galanin agonist by causing contraction of longitudinal muscle strips of the human colon in vitro (Katsoulis *et al.* (1996) Scand. J. Gastroenterol. **31** 446-451). Further, M15 and Gal(1-14)-[Abu8]SCY-I, acting as galanin agonists, both evoked concentration-dependent contractions of gastric smooth muscle strips (Korolkiewicz *et al.* (1996) Pharmacol. Res. **33** 361-365).

10. In insulin-producing RIN m5F cells, M15 and M35 both produce a biphasic response in calcium levels identical to galanin, demonstrating that both chimeric peptides act in this system as galanin agonists (Fridolf & Ahren (1993) Biochem. Biophys. Res. Commun. **191** 1224-1229; Kask *et al.* (1995) Regul. Pept. **59** 341-348). Similarly, a later study showed that M40 acts as a galanin agonist by stimulating glucose-induced insulin release from isolated mouse pancreatic islets (Bartfai *et al.* (1993) Proc. Natl. Acad. Sci. U.S.A. **90** 11287-11291). M40 also acts as a galanin agonist in the spinal cord (Xu *et al.* (1995) Br. J. Pharmacol. **116** 2076-2080). In addition, two N-terminally extended forms of galanin, galanin-(-7-29) and galanin-(-9-29) had also been shown to have agonistic properties on spinal flexor reflex excitability in decerebrate, spinalized, unanesthetized rats (Bedecs *et al.* (1994) Eur. J. Pharmacol. **259** 151-6).

11. Other assays to determine if a compound is a galanin agonist include the technique described by Botella *et al.* (1995) in the journal Gastroenterology **108** 3-11, in which they showed that galanin is an agonist at two types of receptor in intestinal smooth muscle where it contracts or relaxes the tissue.

12. Another way of identifying whether or not a compound was a galanin agonist would have been to examine its effects on the cholinergic control of vasculature tone in the anaesthetized rat, as reported by Barblivien *et al.* (1995) in the journal Neuroreport **6** 1849-1852. In this respect the agonist effect of galanin is to inhibit the vasodilatory cholinergic input.

13. It can been seen from the above studies that a variety of assays have been described which would allow a person of ordinary skill in the art to readily determine whether a compound is a galanin agonist and is, therefore, suitable for use in the method according to claim 18 of the patent application.

14. In summary, I believe that a person of ordinary skill in the art would have been able readily to identify galanin agonists at the priority date of the application and to identify compounds for use in the method according to claim 18 of the patent application.

15. In relation to the rejection of claim 18 for being obvious in light of Luo *et al.* ("Luo") and Zhang *et al.* ("Zhang"), I have also reviewed each of these documents. It seems that the Examiner considers that a compound which is effective in the amelioration of neuropathic pain that occurs as a result of nerve injury (as taught by Luo and then hypothesized by Zhang in primates) would have been obvious to the skilled person as having an effective use in the treatment of nerve damage by nerve regeneration.

16. There were a large number of drugs available for the treatment of chronic neuropathic pain which have no known effect on nerve regeneration. Examples include morphine and other opioids; aspirin; non-steroidal anti-inflammatory agents (NSAIDs); antidepressants such as Amitriptyline or Imipramine; and anti-epileptics such as Tegretol. Furthermore, there is common clinical experience that when drug treatment with these agents is halted, the pain returns, arguing against any restoration of the damaged nerve.

17. Studies have shown that:-

Morphine inhibits facial nerve regeneration (Sinatra, R. S. and Ford, D. H. (1979) Brain Res. **175** 315-25);

The antidepressant Imipramine has no effect on peripheral nerve regeneration in-vivo (De Medinaceli L. *et al.* (1986) Exp. Neurology **94** 788-90).

18. Therefore, in my view, there was nothing in the existing literature to make one of ordinary skill in the art think that chronic treatment of neuropathic pain (arising from nerve injury or damage), irrespective of the cause or the drug used, would promote nerve regeneration. Indeed, there was some data at the priority date of the current application to allow the opposite hypothesis to be proposed. Subsequent publications have supported this, for example Sabouni, F., *et al.* (Biochem. Biophys. Res. Commun. (1998) **248** 165-7) which reported that aspirin delays nerve outgrowth from cultured DRG neurons.

19. All of the existing data at the priority date of the current application was focused on treating pain and not on stimulating nerve regeneration. These two fundamental patho-physiological processes are very different and most, if not all, of the anti-pain drugs listed above act at the level of the spinal cord and brain to reduce electrical and chemical excitability and thus reduce pain transmission. In contrast, drugs that stimulate nerve regeneration do so at the level of the dorsal root ganglion (DRG) and/or the site of nerve injury.

20. For these reasons, it is my view that the skilled person would not have found it obvious to invent a method for the treatment of peripheral nerve damage (or of peripheral sensory neuropathy) in a subject comprising the step of administering a galanin agonist to the subject, wherein the peripheral nerve damage (or peripheral sensory neuropathy) is treated by nerve regeneration.

21. I further declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code; and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: October 31st 2005

Professor Anthony H. Dickenson

EXHIBIT B

First Declaration by Professor Zigmond signed June 28, 2004

ATTORNEY DOCKET NO. 23016.0002US
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of

Wynick, David

Application No. 09/230,463

Filing Date: January 22, 1999

For: "GALANIN"

Art Unit: 1647

Examiner: Gucker, S.

Confirmation No. 4323

DECLARATION UNDER 37 C.F.R. § 1.132

1. I am Professor in the Department of Neurosciences at Case Western Reserve University, Ohio, USA.

2. I have been associated with teaching and research in the subject of Neurosciences for almost thirty years and have published approximately 100 peer-reviewed papers, review articles and chapters during this time. Examples of these publications together with details of my education are given in the short version of my *curriculum vitae* which is attached and shown as Exhibit A.

3. My research relates to neurochemical plasticity in adult neurons. In recent years, my laboratory has focused on the molecules and cells involved in altering neuronal gene expression in response to axonal injury. The galanin peptide has been one molecule of long-standing interest and a major focus of my laboratory.

4. I am familiar with the work of David Wynick in the field of galanin and nerve regeneration.

5. I have reviewed the specification of US Patent Application Serial No. 09/230,463 ("the patent application"). I understand the technology described in the patent application. In

particular, I have reviewed claim 18 of the patent application and its meaning is clear to me.

6. I have also reviewed the publication "The effects of pretreatment with tachykinin antagonists and galanin on the development of spinal cord hyperexcitability following sciatic nerve section in the rat" by Luo, L. and Wiesenfield-Hallin, Z. (1995) Neuropeptides 28 161-166 ("the Luo publication"). I understand the experiments described in that publication and the implications of the data resulting from those experiments.

7. The Luo publication demonstrates that galanin inhibits spinal cord electrical hyperexcitability following nerve section (axotomy) when administered directly into the space surrounding the bottom part of the spinal cord (intrathecal administration to the lumbar enlargement). Such spinal cord excitability, especially after injury, is thought to be related to the development of chronic pain states and blockade of electrical discharge reduces neuropathic pain behaviour. The Luo publication deals exclusively with neuropathic pain behaviour, not with peripheral nerve damage as claimed in the patent application. Peripheral nerve damage is in no way synonymous with chronic neuropathic pain. Further, the actions of galanin when administered directly to the spinal cord (which is part of the central nervous system) to inhibit chronic pain behaviour, in no way implies or predicts regeneration at the level of the sciatic nerve (which is part of the peripheral nervous system).

8. There is no indication in the Luo publication that the treatment of chronic pain is in any way predictive of regeneration of an injured peripheral nerve or, indeed, in the spinal cord. There is no suggestion in the Luo publication that a galanin agonist would be effective in the treatment of peripheral nerve damage in a subject.

9. The patent application provides evidence that galanin has the ability to promote functional nerve regeneration. However, it is highly unlikely that such regeneration would have occurred in the animals used in the experiments of the Luo publication since galanin was administered directly onto the spinal cord. It is most unlikely that the galanin

peptide would reach, or be transported to, the damaged end of the sciatic nerve (which is the closest component of the peripheral nervous system) and thus would not be expected to alter peripheral nerve regeneration. Further, the experiment was begun one hour after spinal transection and, as shown in Figure 3, the galanin experiment was carried out over 150 minutes from the start of the experiment. Therefore, the rats were utilised in the experiment for a maximum of 3.5 hours after decerebration and spinal cord section. This short time period would not be sufficient for functional nerve regeneration to occur in response to the presence of galanin.

10. The results contained in the patent application indicate that, in wild type mice, functional nerve regeneration does not reach significant levels until at least several days after nerve injury (Figure 5). In addition, functional recovery in wild type mice is not complete until approximately three weeks after nerve injury (Figure 6), indicating that effective nerve regeneration and therefore treatment of peripheral nerve damage is a prolonged process.

11. In summary, it is my opinion that the Luo publication does not give any incentive to the person of ordinary skill in the art to attempt a method for the treatment of peripheral nerve damage in a subject in need of such treatment, the method comprising the step of administering to the subject an amount of a galanin agonist effective to treat peripheral nerve damage as recited by the claims of the patent application.

12. I further declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: June 28, 2004

Richard E. Zigmond

Professor Richard E. Zigmond

EXHIBIT A

BIOGRAPHICAL SKETCH	
NAME RICHARD E. ZIGMOND	POSITION TITLE PROFESSOR OF NEUROSCIENCES

EDUCATION/TRAINING INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Harvard College, Cambridge MA	BA cum laude	1962-66	Biology
Rockefeller U., NY, grad. stud. with Bruce McEwen	Ph.D.	1966-71	Neuroscience
Rockefeller U., NY, postdoc. with Don Pfaff		1971-72	Neuroendocrinology
U. of Cambridge, UK, postdoc. with Leslie Iversen		1972-75	Neurochemistry
Harvard Medical School/Children's Hospital, (Sabbatical with Michael Greenberg)		2000-01	Molecular Biology

Positions and Honors

Appointments: Assist. Prof. (1975-81), Assoc. Prof. (1981-89) of Pharmacology, Harvard Medical School; Instructor in Neurobiology of Behavior at Cold Spring Harbor Lab. (1979-82); Prof. of Neurosci., Case Western Reserve Univ. (CWRU) School of Medicine (1989-present); Instructor in Neurobiology at Marine Biology Lab. (1981-84); Program Director, NIH Postdoctoral Training Program in Devel. Neurol. (1981-89; Harvard Med. Sch.); Instructor on Review and Update in Neurobiology for Neurosurgeons (1984, 86, 88); Chair, Committee on Appointments and Tenure, Department of Neurosciences, CWRU (1991-present); Chair, Gordon Conference on Neural Plasticity (1991); Program Committee, Society for Neuroscience (1991-93); Acting Chair, Department of Neurosciences, 1992-93).

Fellowships and Special Grant Awards: Pop. Council Fellowship in Mammalian Reproduction (1971-72); British-American Heart Found Fellowship (1972-73); Sloan Found. Fellowship in Neurochem. (1972-74); Klingenstein Fellowship in the Neurosciences (1987-1990); Mellon Found. Faculty Award (1976-1977); NIMH Research Scientist Development Award (1977-87); Javits Neuroscience Investigator Award (1987-94); NIMH Research Scientist Award (1987-94).

Grant Review Committees: External review committee for the Lab. of Developmental Neurobiology NICHD (1985, 1990); Study Section for Tobacco-Related Disease Research Program of the Univ. of California (1993, 1994); Ad hoc Reviewer, Neurology C Study Section (Neuro C; 1995, 1996); Member, Neurological Sciences 1 Study Section (NLS1) and Molecular Developmental and Cellular Neurosciences Study Section (MDCN7; 1996-2000); Reviewer of Research at the Burke Medical Research Institute (1998).

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Editorial Boards: *J. Neurosci. Meth.* (1978-1998), *J. Neurosci.* (1985-91), *Ann. Rev. Neurosci.* (1986-90), *TINS* (1986-90), *New Biol.* (1989-91), *Adv. in Neurosci.* (1989-present), *Neuroscience* (1996-present), *J. Mol. Neurosci.* (1997-present), *Autonomic Neuroscience: Basic and Clinical* (formerly *J. Auton. Nerv. Syst.* (1998-), *NeuroSignals* (formerly *Biological Signals and Receptors* (2001-present).

Selected peer-reviewed publications since 1998.

1. Rao MS, Sun Y, Escary JL, Perreau J, Patterson PH, Zigmond RE, Brulet P, Landis SC. Leukemia inhibitory factor mediates an injury response but not a developmental transmitter switch in sympathetic neurons. Neuron 11:1175-1185, 1993.
2. Sun Y, Rao MS, Zigmond RE, Landis SC. Regulation of vasoactive intestinal peptide expression in sympathetic neurons in culture and after axotomy: The role of cholinergic differentiation factor/leukemia inhibitory factor. J Neurobiol 25:415-430, 1994.
3. RC, Shadiack AM, Bennett TA, Sedwick CE, Zigmond RE. Changes in the macrophage population of the rat superior cervical ganglion after postganglionic nerve injury. J Neurobiol 27:141-153, 1994.
4. Sun Y, Landis S, Zigmond R. Signals triggering the induction of leukemia inhibitory factor in sympathetic superior cervical ganglia and their nerve trunks after axonal injury. Mol Cell Neurosci 7:152-163, 1996.
5. Hyatt-Sachs H, Bachoo M, Schreiber R, Vaccariello SA, Zigmond RE. Chemical sympathectomy and postganglionic nerve transection similarly increase galanin and VIP mRNA but not the peptides themselves. J Neurobiol 30:543-555, 1996.
6. Sun Y, Zigmond RE. Leukemia inhibitory factor induced in the sciatic nerve after axotomy is involved in the induction of galanin in sensory neurons. Europ J Neurosci 8:2213-2220, 1996.
7. Zigmond, RE. Retrograde and paracrine influences on neuropeptide expression in sympathetic neurons after axonal injury. In: Cytokines and the CNS: Development, Defenses and Disease (RM Ransohoff, EN Benveniste, Eds) CRC Press, Boca Raton, pp. 169-186, 1996.
8. Zigmond RE, Hyatt-Sachs H, Mohney RP, Schreiber RC, Shadiack AM, Sun Y, Vaccariello SA. Changes in neuropeptide phenotype after axotomy of adult peripheral neurons and the role of leukemia inhibitory factor. Perspec Dev Neurobiol 4:75-90, 1996.
9. Zhou Y, Deneris E, Zigmond RE. Differential regulation of levels of nicotinic receptor subunit transcripts in adult sympathetic neurons after axotomy. J Neurobiol 34:164-178, 1998.
10. Shadiack AM, Zigmond RE. Galanin induced in sympathetic neurons after axotomy is anterogradely transported toward regenerating nerve endings. Neuropeptides 32:257-264, 1998.
11. Shadiack AM, Vaccariello SA, Sun Y, Zigmond RE. Nerve growth factor inhibits sympathetic neuron's response to an injury cytokine. Proc Natl Acad Sci USA 95:7727-7730, 1998.
12. Mohney RP, Zigmond RE. Vasoactive intestinal peptide enhances its own expression in sympathetic neurons after injury. J Neurosci 18:5285-5293, 1998.
13. Zigmond RE, Mohney RP, Schreiber RC, Shadiack, AM, Sun Y, Vaccarriello SA, Zhou Y. Plasticity in gene expression in adult sympathetic neurons after axonal damage. Adv in Pharmacol 42:899-903, 1998.
14. Nagamoto-Combs K, Vaccariello S, Zigmond RE. The levels of LIF mRNA in a Schwann cell line are regulated by multiple second messenger pathways. J Neurochem 72:1871-1881, 1999.
15. Mohney RP, Zigmond RE. Galanin expression is decreased by cAMP-elevating agents in cultured sympathetic ganglia. NeuroReport 10:1221-1224, 1999.

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16. Rittenhouse A R., Zigmond RE. The role of N- and L-type calcium channels in the depolarization-induced activation of tyrosine hydroxylase and release of norepinephrine by sympathetic cell bodies and nerve terminals. J Neurobiol 40:137-148, 1999.
17. Boeshore KL, Luckey CN, Zigmond RE, Large TH. TrkB isoforms with distinct neurotrophin specificities are expressed in predominantly non-overlapping populations of avian dorsal root ganglion neurons. J Neurosci 19:4739-4747, 1999.
18. Ip NY, Zigmond RE. Synergistic effects of muscarinic agonists and secretin or vasoactive intestinal peptides on the regulation of tyrosine hydroxylase activity in sympathetic neurons. J Neurobiol 42:14-21, 2000.
19. Bibevski S, Zhou Y, McIntosh J, Zigmond R, Dunlap M. Functional nicotinic acetylcholine receptors that mediate ganglionic transmission in cardiac parasympathetic neurons. J. Neurosci 20:5076-5082, 2000.
20. Zigmond RE. Neuropeptide action in sympathetic ganglia: Evidence for distinct functions in intact and axotomized ganglia. Ann N Y Acad Sci.921:103-108, 2000.
21. Shadiack A, Sun Y, Zigmond R. Nerve growth factor antiserum induces axotomy-like changes in neuropeptide expression in intact sympathetic and sensory neurons. J. Neurosci. 21:363-371, 2001.
22. Zhou Y, Deneris E, Zigmond R. Nicotinic acetylcholine receptor subunit proteins alpha7 and beta4 decrease in the superior cervical ganglion after axotomy. J Neurobiol. 46: 178-192, 2001.
23. Schreiber RC, Krivacic K, Kirby, B, Vaccariello SA, Tani M, Ransohoff RM, and Zigmond RE. Monocyte chemoattractant peptide- 1 (MCP-1) is rapidly expressed by sympathetic ganglion neurons following axonal injury. NeuroReport 12:601-606, 2001.
24. Zigmond RE. Can galanin also be considered as growth-associated protein 3,2? TINS 24:494-496, 2001.
25. Takasu, AM. Dalva MB, Zigmond R, Greenberg, ME. Science. EphB receptors modulate NMDA receptor-dependent calcium influx and gene expression. Science 295: 491-495, 2002.
26. Schreiber RC, Boeshere K, Vaccariello SA, Shadiack, Zigmond RE. A comparison of the changes in the non-neuronal cell populations of the superior cervical ganglia following decentralization and axotomy. J. Neurobiol. 53: 68-79, 2002.
27. Zigmond R.E. Plasticity in the autonomic nervous system: Responses of adult sympathetic neurons to injury. In: Handbook of Autonomic Nervous System in Health and Disease (J Licinio and L Bolis, Eds) Marcel Dekker, New York, pp. 167-184, 2002.

Research Support

Sponsor: National Institutes of Health **Award Number:** NS12651
Dates: 7/1/2003-6/30/2004 **Amounts:** Current Direct Costs: 0
Title: Experience and the Neurochemistry of the Synapse
Percent Effort: 35%
Brief description of the project: To identify the transmitter responsible for the non-cholinergic activation of TH in the SCG after preganglionic nerve stimulation, determine whether such nerve stimulation alters neuropeptide expression, determine whether PACAP and VIP are involved in feedback mechanisms regulating their own expression, examine the signals triggering the changes in nAChR receptor subunit mRNA expression in axotomized SCG neurons, determine if changes occur at the receptor level, and ask whether a phenomenon comparable to "disuse supersensitivity" is seen in these receptors as a result of changes in afferent nerve stimulation.

EXHIBIT A

Sponsor: National Institutes of Health **Award Numbers:** NS17512
Dates: 5/15/03-4/30/07 **Amounts:** Current Direct Costs: $237,500
Title: Recovery of Function after Neural Damage
Percent Effort: 35%
Brief description of the project: To determine the cellular and molecular changes that occur in peripheral neurons in the context of regeneration. We have been notified by the NINDS that this application will be refunded.

EXHIBIT C
Second Declaration by Professor Zigmond signed June 1, 2005

ATTORNEY DOCKET NO. 23016.0002US
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of	)	
	)	
Wynick, David	)	Art Unit: 1647
	)	
Application No. 09/230,463	)	Examiner: Gucker, S.
	)	
Filing Date: January 22, 1999	)	Confirmation No. 4323
	)	
For: "GALANIN"	)	

DECLARATION UNDER 37 C.F.R. § 1.132

1. I am Professor in the Department of Neurosciences at Case Western Reserve University, Ohio, USA.

2. I have been associated with teaching and research in the subject of Neurosciences for almost thirty years and have published approximately 100 peer-reviewed papers and 25 review articles and chapters during this time. Examples of these publications together with details of my education are given in the short version of my *curriculum vitae* which is attached and shown as Exhibit A.

3. My research relates to neurochemical plasticity in adult neurons. In recent years, my laboratory has focused on the molecules and cells involved in altering neuronal gene expression in response to axonal injury. The galanin peptide has been one molecule of long-standing interest and a major focus of my laboratory.

4. I am familiar with the work of David Wynick in the field of galanin and nerve regeneration.

5. I have reviewed the specification of US Patent Application Serial No. 09/230,463 ("the patent application"). I understand the technology described in the patent application. In

particular, I have reviewed claim 18 of the patent application and its meaning is clear to me.

6. I have also reviewed the publication "The effects of pretreatment with tachykinin antagonists and galanin on the development of spinal cord hyperexcitability following sciatic nerve section in the rat" by Luo, L. and Wiesenfield-Hallin, Z. (1995) Neuropeptides **28** 161-166 ("the Luo publication"). I understand the experiments described in that publication and the implications of the data resulting from those experiments.

7. In addition, I have reviewed the Office Action mailed on 10th March 2005, particularly item 5 of the Action in which the Examiner rejected claims 18 and 25-26 under 35 U.S.C. 103(a) as being unpatentable over the Luo publication in view of Zhang *et al.* (J. Neurocytology (1993) **22** 342-381).

8. The Luo publication demonstrates that galanin inhibits spinal cord electrical hyper-excitability for 60 minutes following nerve section (see Figure 3), at which point the animals were sacrificed. Galanin was administered 30 minutes before the nerve injury as a one-off bolus injection of 2.4nM (low dose) directly into the space surrounding the bottom part of the spinal cord (intrathecal (IT) administration into the lumbar enlargement, see page 163, paragraph headed "Effect of galanin" and Figure 3 of the Luo publication). The Luo publication deals exclusively with neuropathic pain and spinal cord excitability, not with peripheral nerve regeneration as claimed in the patent application. The mechanism by which the galanin rapidly alters pain activity is most likely by direct modulation of spinal cord neuronal firing rather than at the level of the dorsal root ganglion (DRG).

9. When damage or injury to sensory or motor nerves (in this case the sciatic nerve) occurs, this triggers a cascade of molecular events within the cell bodies of that nerve, in this case the DRG, which in turn attempts to repair the damage and restore the normal function of the nerve, so-called nerve regeneration. The Protein Kinase C (PKC) and MAP Kinase (MAPK) intra-cellular signalling cascades have both been shown to up-

regulate after nerve injury and are vital for nerve regeneration (Klinz & Heumann (1995) J. Neurochem. **65** 1046-53; Kiryu et al. (1995) Brain Res. Mol. Brain Res. **29** 147-56). At the 1996 priority date of the patent application, no published literature existed to indicate that galanin speeded up nerve regeneration, nor that it activated PKC or MAPK.

10. For regeneration to have occurred in the animals used in the experiments documented in the Luo publication during the 90 minute period before the animals were sacrificed, the galanin would have had to gain access to the cell bodies in the DRG, since this is where the intra-cellular pro-regenerative machinery resides (Terenghi (1994) J. Anatomy (Pt I) 1-14). The only way that galanin, when applied to the space surrounding the bottom part of the spinal cord, could have reached the cell bodies of the sensory neurons in the DRG which is where "...the preliminary beginnings of regeneration..." would have occurred, is by direct uptake of the galanin by the nerve terminals in the dorsal horn of the spinal cord. The cell bodies of the sensory neurons of the DRG lie outside the central nervous system (CNS), whilst the spinal cord is part of the CNS. The cerebrospinal fluid (CSF) that bathes the spinal cord is not in contact with the DRG and thus galanin could not have reached the DRG by passive diffusion.

11. There are well documented and active transport mechanisms in sensory neurons that move proteins from the nerve terminals of the spinal cord or the ends of the peripheral axons of the sciatic nerve to the cell bodies in the DRG, termed retrograde transport. A number of these retrograde transport mechanisms for Nerve Growth Factor (NGF) and Horseradish Peroxidase (HRP) have been extensively studied and characterized. There is good agreement between these published papers that the rate at which these retrograde transport processes move proteins from the rat dorsal horn of the spinal cord to the cell body in the DRG is a maximum rate of 7.5 mm/hour (range 2.5 – 7.5 mm/hr, Yip and Johnson, Jr. (1986) J. Neurocytol. **15** 789-98; Richardson and Riopelle. (1984) J. Neurosci. **4** 1683-9).

12. Michael et al. (J. Neurosci. (1997) **17** 8476-8490) found the nerve root of adult male Wistar rats (200-400g body weight) to be 17 mm in length. Similarly, Baba et al. (Baba et al. (1999) J. Neurosci. **2** 859-867) found the dorsal root to be between 18-20 mm in

length in adult male Sprague-Dawley rats weighing 300-350g. Based on this, assuming the maximum rate of retrograde transport of galanin from the dorsal horn of the spinal cord to the DRG is 7.5 mm/hr and the length of the nerve root between the dorsal horn of the spinal cord and the DRG is at least 17mm in length, then the galanin would only have been transported 11.25 mm in the 90 minutes after galanin was administered before the animals were sacrificed (i.e. about two thirds of the way to the DRG). Galanin could not, therefore, have even begun to affect regeneration in the DRG cell bodies by the time the experiment was terminated.

13. In addition, the concentration of galanin would have immediately and rapidly begun to fall straight after the administration of the bolus injection of 2.4nM galanin into the lumbar enlargement of the spinal cord. This would have occurred within seconds, as the galanin would immediately be diluted by the CSF and almost immediately would also have started to be degraded by proteolytic enzymes in the CSF (Bedecs et al. (1995) Neuropeptides **29** 137-43). Therefore, even in the highly unlikely event that a small proportion of the galanin that was administered by bolus-dose to the spinal cord did reach the DRG by retrograde transport, the final dose would be substantially lower (in the sub-nanomolar range) than the originally administered IT (page 163 and Figure 3 of the Luo publication). In contrast, the dose of galanin demonstrated to stimulate nerve outgrowth from sensory neurons by direct application in cell culture to the DRG cell body is 100nM galanin (Mahoney et al. (2003) J. Neurosci. **23** 416-421). In light of this, the effective concentration of galanin that would have reached the DRG would have been at least 100-fold lower than that necessary to stimulate regeneration, again making it highly unlikely that galanin could have even begun to affect regeneration in the DRG cell bodies by the time the experiment was terminated.

14. For these reasons, it is my view that one of ordinary skill in the art would have no incentive, on reading the Luo publication, to imagine that galanin-induced nerve regeneration of the severed sciatic nerve would have begun during the time period utilized in the experiments of Luo et al. In addition, in light of the Luo et al. disclosure and on reading the disclosure in Zhang et al. that galanin may be suitable for use as an

analgesic in humans, one of ordinary skill in the art would have no motivation to administer a galanin agonist in a method for the treatment of peripheral nerve damage, wherein the peripheral nerve damage is treated by nerve regeneration, as claimed in claim 18 as amended with the most recently filed Applicant's response.

15. I further declare that all statements made herein of my own knowledge and belief are true and that all statements made on information and belief are believed to be true, and further, that the statements are made with the knowledge that willful false statements are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 6/1/05

Professor Richard E. Zigmond